THE HAEMATOZOA OF AUSTRALIAN MARINE TELEOSTEI.

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(Six Text-figures.)

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Introduction.

No blood parasites have been recorded from Australian marine fish, which is surprising in view of their common occurrence in other parts of the world. The "ring bodies" described by Johnston and Cleland (1909) from the red blood cells of *Monocanthus* sp. may possibly be protozoa, but their interpretation, that they are centrosomes, is more probably correct. The same authors later (1910) listed 46 marine Teleostei examined with negative results. The findings here recorded serve to bring the Australian haematozoal fauna into line with that of other regions.

While many species of Trypanosomes and Haemogregarines are known from marine fish, blood inhabiting Trypanoplasms have, so far as we can determine, only been recorded hitherto from freshwater fish.

It is a pleasure to acknowledge our indebtedness to the late Mr. A. R. McCulloch of the Australian Museum, Sydney, who has identified most of our host species. The nomenclature followed here is that given in his "Check List" (McCulloch, 1922).

Part of this work was carried out while one of the authors (I. M. Mackerras) was the holder of the John Coutts and Science Research Scholarships in the University of Sydney.

Methods.

Smears were made from the heart blood as soon as the fish was caught, dried as rapidly as possible and stained on our return home either with Leishman's Stain or with a solution of "Soloid" Eosin-Azur in pure methyl alcohol. Both methods were used without previous fixation and the latter gave particularly good results, especially if the staining time was extended (3-5 minutes in undiluted, and 15-45 minutes in diluted stain).

Conditions were such as to preclude the use of wet-fixation methods, which is to be regretted, as many important details of morphology are not seen satisfactorily in dried films. Having in mind the use which may be made of parasites in determining host relationships, we have consequently refrained from discussing the affinities of the parasites here described.

Fish blood is remarkable for the extreme rapidity with which it clots, either in the body after death, or on being drawn from the living animal. Films must therefore be made as soon as the fish is caught, and haste is necessary once the heart is opened in order to obtain even three or four satisfactory preparations.

Material.

The material was collected at Sydney and at Broken Bay, near Sydney, N.S.W., during the past four years at such times as opportunity offered. Fish were examined at all seasons, the majority, however, during the winter, and parasites were found in the months of January, May, June and November. Two hundred fish belonging to 31 species and 27 genera have been examined. The following list gives those species which have been searched with negative results, together with the number of individuals of each species examined. It is to be noted that the infections found have always been scanty, consequently we may have missed an occasional parasite in those smears classified as negative, although each was submitted to a prolonged and careful search.

Acanthistius serratus (Cuv. and Val.). Wirrah	6
Atypichthys strigatus (Gunth.). Mado	1
Cantherines ayraudi (Quoy and Gaimard). Leather-jacket	17
Cantherines hippocrepis (Quoy and Gaimard). Variable Leather-jacket	2
Cantherines sp. (Young). Leather-jacket	5
Cnidoglanus megastomus (Rich.). Estuary Cat Fish	2
Dactylopagrus morwong (Ramsay and Ogilby). Morwong	1
Dicotylichthys punctulatus Kaup. Porcupine Fish	4
Enoplosus armatus (Shaw). Old Wife	3
Hippocampus novaehollandiae Steind. Sea Horse	11
Leptocephalus labiatus (Castelnau). Conger Eel	1
Lotella callarias Gunth. Beardie	1
Neoplatycephalus macrodon Ogilby. Tiger Flathead	1
Ophthalmolepis lineolatus (Cuv. and Val.). Maori	4
Pagrosomus auratus (Forster). Snapper	27
Platycephalus fuscus Cuv. and Val. Dusky Flathead	8
Pseudolabrus gymnogenis (Gunth.). White-spotted Parrot Fish	6
Pterygotrigla polyommata (Rich.). Latchet	1
Sciena antarctica Castelnau. Jewfish	11
Scomber australasicus Cuv. and Val. Mackerel	2
Scorpaena cardinalis Rich. Red Rockcod	5
Scorpis lineolatus Kner. Sweep	11
Spheroides hamiltoni (Rich.). Common Toado	6
Trachichthodes affinis (Gunth.). Nannygai	2
Trachurus declivus (Jenyns). Yellow-tail	1
Upeneichthys porosus (Cuv. and Val.). Blue-striped Red Mullet	2
Verreo oxycephalus (Bleeker). Pig-Fish	1
Total	142

Parasites have been found in the following:—

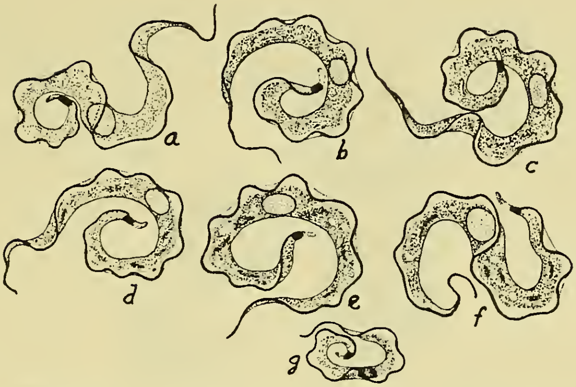
Aulopus purpurissatus Rich. Sergeant Baker. (Fam. Aulopidae, Ord. Iniomi.). 8 examined, Trypanosomes in 1, Haemogregarines in 1.

Gilbertia annulata (Gunth.). Banded Sea Perch. (Fam. Serranidae, Sec. Perciformes, Subord. Percoidea, Ord. Percomorphi.). 3 examined, Haemogregarines in 1.

Gilbertia semicincta (Cuv. and Val.). Half-banded Sea Perch. 38 examined, Trypanosomes in 3, Haemogregarines in 4.

Parma microlepis Gunth. White-ear. (Fam. Pomacentridae, Sec. Pomacentriformes, Subord. Percoidea, Ord. Percomorphi.). 9 examined, Trypanosomes in 1, Trypanoplasms in 3, Haemogregarines in 7.

The total number of individuals infected was 15, or 7.5% of all fish examined.



Text-figure 1. *Trypanosoma pulchra,* n. sp.; *a, b* and *c* from *Gilbertia semicincta* (Cuv. and Val.); *d, e* and *f* from *Parma microlepis* Gunth.; *g,* small form from *Gilbertia semicincta* (Cuv. and Val.). × 1180.

Haemoflagellates.

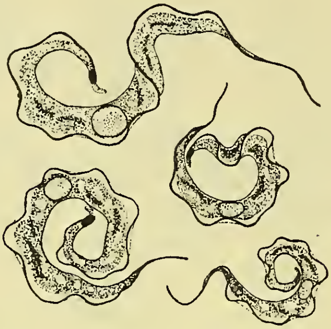
TRYPANOSOMA PULCHRA, n. sp.

Hosts: *Gilbertia semicincta* (Cuv. and Val.) and *Parma microlepis* Gunth. Present in 3 out of 38 of the former and 1 out of 9 of the latter. Sydney and Broken Bay, N.S.W. January, May and June.

An average individual from the type slide is shown in Fig. 1*a* and has the following measurements: Length of body 48.3μ, diameter of body at level of trophonucleus 3.5μ, kinetonucleus is 1.7μ from aflagellar end and measures 1.6μ by 0.8μ, trophonucleus is 22.5μ from aflagellar end and measures 4.0μ by 2.9μ, diameter of undulating membrane 1.0μ to 1.5μ, free part of flagellum 7.3μ. The body length varies from 57.1μ to 40.8μ, but the relative proportions of the parts and the general appearance of the individuals remain constant within fairly narrow limits, except for two small specimens to be described later. The cytoplasm of the body is rather dense and stains a deep blue with Eosin-Azur. It shows irregular lighter and darker areas and a number of small scattered vacuoles. The aflagellar end is beak-shaped and stains a very pale blue. The undulating membrane is clear and takes a pale pink stain. The flagellum appears to arise directly from the deep red kinetonucleus, there being no separate basal granule visible. The trophonucleus stains uniformly pink and shows no sign of a karyosome or of chromatinic granules.

In one infection (*G. semicincta* No. 26), two very small individuals were seen along with the ordinary form (Fig. 1*g*). Their lengths are 27.2μ and 26.5μ and they differ from the common form in that the flagellum is relatively longer, the undulating membrane is relatively wider (being in both cases about the same as in the larger type), and in the condition of the trophonucleus which shows a number of dark granules scattered throughout, but rather more numerous at the ends. What relation these bear to the common type, or whether they are possibly a distinct species, we are at present unable to say. Similarly, we cannot decide positively whether the infections from the two hosts represent one or two species. Though the hosts are not very closely related, they are found together in the same situation on rocky bottoms and the parasites are morphologically indistinguishable. Consequently, we see no valid reason for separating them at present.

Type slide (*G. semicincta* No. 35*g* in our series) in the Australian Museum, Sydney.



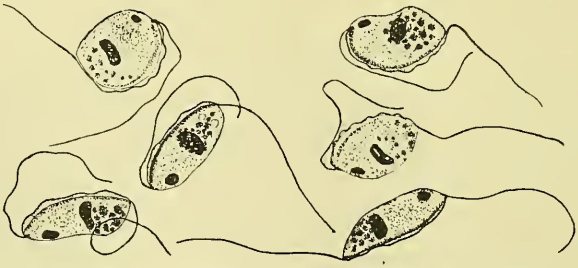
Text-figure 2. *Trypanosoma aulopi*, n. sp. × 1180.

TRYPANOSOMA AULOPI, n. sp.

Host: *Aulopus purpurissatus* Rich. Present in 1 out of 8. Sydney, N.S.W. May.

This species, of which we have but one scanty infection, is separated from *T. pulchra*, nov., because of its very marked pleomorphism. In general body form, proportions, and staining reactions it is similar to the common larger form of *T. pulchra*, nov., but individuals of widely different sizes are encountered with equal frequency. The following are the body lengths (in μ) of nine consecutive individuals arranged in order of size:—57.1, 53.0, 50.3, 47.5, 44.8, 36.3, 33.5, 33.5, 29.1. Nothing was found quite as small as the small specimens from *G. semicincta*. The host belongs to the Iniomi, an order widely separated from the Percomorphi, to which the hosts of *T. pulchra*, nov., belong. It is, however, also a rock fish living in a not very different habitat and there are definite possibilities of cross infection occurring. Taking everything into consideration, and having in mind the usual practice amongst haematozoologists, it seems most reasonable in the present state of our knowledge to propose a separate name for this species, leaving it for future work with much more extensive series from all three hosts to decide the identity or otherwise of the various forms.

Type slide (*A. purpurissatus* No. 1 of our series) in the Australian Museum, Sydney.



Text-figure 3. *Trypanoplasma parmae*, n. sp. × 1180.

TRYPANOPLASMA PARMAE, n. sp.

Host: *Parma microlepis* Gunth. Present in 3 out of 9. Sydney, N.S.W. November.

Owing to the soft and mobile nature of the body most of the parasites become somewhat distorted in dried films and the following description is based on those specimens which had obviously retained their natural shape more or less completely. This species is monomorphic and of a somewhat broad type, varying from 12.5μ long by 3.8μ wide to 14.7μ long by 5.0μ wide. The cytoplasm stains a faint blue and is finely and irregularly granular. Anteriorly it is pale and indistinctly vacuolated, whilst behind the trophonucleus it is denser and contains numerous round or irregular chromatoid bodies, 0.25μ to 1.25μ or occasionally more in diameter; these sometimes extend round the anterior margin of the trophonucleus. Along the full length of the dorsal edge of the body, and marking the origin of the undulating membrane, is a narrow zone of densely packed fine granules which stain a reddish colour with Eosin-Azur.

The trophonucleus, which is situated behind the middle of the body, is reniform or occasionally oval in shape, measures 3.7μ to 3.0μ by 1.5μ to 2.3μ and is fairly densely packed with large irregular chromatinic masses. The kinetonucleus is a large oblong or oval mass, from 1.7μ to 2.0μ long by 1.0μ to 1.3μ wide, and is situated close to the ventral surface about 2μ from the anterior end of the body; it stains an intense reddish-black and rarely gives any indication of internal organization. The blepharoplast is a minute darkly stained granule, which usually appears single but is occasionally clearly double; it lies close to the anterior extremity of the body. No definite rhizoplast could be made out in our preparations. The anterior free flagellum measures from 18.0μ to 25.0μ in length. The posterior flagellum is attached to the body by a very narrow undulating membrane which is little more than 0.25μ wide; its free terminal part measures 18.0μ to 26.8μ.

Many of the specimens in our slides were rounded and some showed irregular bulgings as if about to burst. Such distorted examples are from 9.5μ to 11.1μ long by 6.9μ to 8.5μ wide, the measurements of other parts being similar to those given above. In one or two specimens a number of clear unstained vacuoles, 1μ to 2μ in diameter, were seen; these had very sharply marked outlines and gave the impression of being signs of impending dissolution rather than of any normal process.

The special interest attaching to this parasite is that, while *Trypanoplasma* (or *Cryptobia*) has long been known as a parasite of invertebrates, of the alimentary tract of frogs and marine fish, and of the blood of freshwater fish, this is, so far as we are aware, the first record of its occurrence in the blood of a marine fish.

Type slide (*P. microlepis* No. 4*d* of our series) in the Australian Museum, Sydney.

Haemogregarines.

HAEMOGREGARINA AULOPI, n. sp.

Hosts: *Aulopus purpurissatus* Rich. and ? *Parma microlepis* Gunth. Present in 1 out of 7 of the former and ? 2 out of 9 of the latter. Sydney, N.S.W. May.

This parasite was present in the same slide as *Trypanosoma aulopi*, nov., and was not nearly so scanty. It is small and is not so uniform in shape as is the case with most Haemogregarines. In certain respects it resembles the little known genus *Pirhaemocyton* Chatton et Blanc, which is parasitic in Geckos.

Three distinct types of parasite are present in this infection, large oval forms, small rounded forms and slender forms. The large oval forms (Fig. 4*a*) are least common, measure 4.5μ by 2.9μ, and occur singly. The outline is definite

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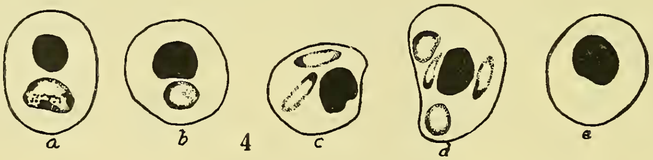
and the cytoplasm clear, except for a peripheral zone which is denser and stains blue; sometimes delicate branching strands of denser blue cytoplasm invade the clear central area. The chromatin is collected for the most part in blocks at the periphery, but strands of linked granules extend in many cases into the central area.

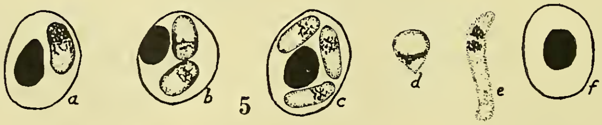
The rounded forms (Fig. 4, *b* and *d*) most frequently occur two in a cell, but single infections are often met with, and once four were seen in one cell. The size seems to bear some relation to the number present in the cell, single infections measuring about 3.3μ by 2.5μ, double infections are slightly smaller, not more than 3.0μ long, usually less, while in the quadruple infection the parasites measured 2.3μ by 1.8μ. The cytoplasm is similar to the larger form, but the chromatin is more densely aggregated into peripheral blocks or bands; one specimen was intermediate between the two forms in size and chromatinic arrangement. It seems likely that the smaller forms are the products of division of the larger, fission being into two, and these may divide again, giving rise to the four very small parasites described above.

The slender form (Fig. 4, *c* and *d*) always occurred in pairs. In one case a pair of the slender form and a pair of the rounded form occupied the same cell (*d*). They were fairly uniform in size, measuring on an average 4.7μ by 1.5μ. Their outline is always more delicate than in the other forms and the chromatin is collected into a block at one end with an occasional granule at the periphery further along the body. Nothing was seen linking these with the other forms, and what stage they represent we cannot say at present, though they may be the gametocytes.

In *Parma microlepis* No. 6*a*, a single cell was found containing four parasites rather closely resembling those shown in Fig. 4*d*, and in *Parma microlepis* No. 7*a*, one cell was also seen containing two short rather shrunken parasites. These are quite possibly *H. aulopi*, nov., but, as no other parasites of this type were found in all our material from *Parma microlepis*, one cannot be sure of the identification.

Type slide (*A. purpurissatus* No. 1 of our series) in the Australian Museum, Sydney.





Text-figure 4. *Haemogregarina aulopi*, n. sp. *a*, *b*, *c* and *d*, different types of infections; *e*, normal host cell. × 1400.
Text-figure 5. *Haemogregarina gilbertiae*, n. sp. *a*, *b* and *c*, intracellular forms; *d* and *e*, vermicules; *f*, normal host cell. × 1400.

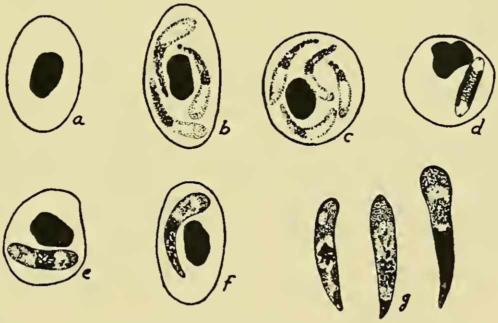
HAEMOGREGARINA GILBERTIAE, n. sp.

Hosts: *Gilbertia semicincta* (Cuv. and Val.) and *Gilbertia annulata* (Gunth.). Present in 4 out of 38 of the former and 1 out of 3 of the latter. Sydney and Broken Bay, N.S.W. January and November.

This species is larger and more uniform in shape than *H. aulopi*, nov. It is usually single, but double infections frequently occur, and once three parasites were found in one cell (Fig. 5*c*). The shape is broadly oval and the size is in general very uniform, being about 4.2μ by 2.5μ. Smaller or larger forms are rare; the smallest individual seen measured 3.5μ by 2.0μ, while a few longer, somewhat slenderer individuals were found which measured up to 5.6μ by 2.2μ. The parasites in single infections were slightly larger than those in double infections in which the members of a pair were almost always of exactly the same size. The cytoplasm is clear and only stains at the periphery. The chromatin is arranged in a loose skein towards one end of the parasite and is sometimes more concentrated in blocks at the sides (Fig. 5*b*); occasionally it is widely spread out in the form of scattered granules.

Two free parasites were seen, the first (Fig. 5*d*) apparently being an early stage in the development of a free vermicule. It measures 4.3μ by 3.4μ and consists of two parts separated by a band of chromatinic granules. The larger part resembles the ordinary intracellular parasite, while the smaller tapering part stains like the fully developed vermicule. This second form (Fig. 5*e*) measures 10.4μ by 1.9μ; its outline is delicate and the cytoplasm stains a pale greyish-blue; the chromatin is collected into two masses of granules towards one end. Both ends are rounded and the body has not the streamline form usual amongst vermicules as exemplified by the parasites shown in Fig. 6*g*. The intracellular parasites could not be differentiated into gametocytes and schizonts.

Type slide (*G. semicincta* No. 27*d* of our series) in the Australian Museum, Sydney.



Text-figure 6. *Haemogregarina parmae*, n. sp. *a*, normal host cell; *b*, *c* and *d*, schizonts; *e* and *f*, intracellular gametocytes; *g*, free gametocytes. × 1400.

HAEMOGREGARINA PARMAE, n. sp.

Host: *Parma microlepis* Gunth. Present in 7 out of 9 examined. Sydney, N.S.W. November and June.

The more numerous and more abundant infections available have enabled us to study this species in greater detail than was possible in the case of the other species described above. Two distinct stages are present in our preparations; they can be allotted with little doubt to the schizogenous and gametogenous generations respectively.

The schizonts occur most frequently three or four in a cell; occasionally single or double infections are to be met with, and very rarely as many as six or seven parasites occupy a single cell, these being probably the result of a double initial infection. Since our infections are never so abundant as to make it probable that double infections are more than a very occasional occurrence, the presumption is that the three or four parasites usually found have originated from a single initial infection. There is usually definite enlargement of the infected cell, the amount of enlargement and the size of the contained parasites probably being an indication of the duration of the infection of that cell. The individual parasites (Fig. 6, *b* and *c*) vary from 6.2μ to 9.6μ long, about 8μ being the commonest, by from 1.2μ to 1.7μ wide. The body is delicate in outline, tapering more towards one end than the other and is usually somewhat curved. The cytoplasm stains a delicate blue, paler towards the centre which, occasionally, has the appearance of a definite oval vacuole. The chromatinic material is aggregated towards the middle with strands and granules extending into the more attenuated end of the body.

The gametocytes are more definite in outline and stouter bodied than the schizonts. When contained within a cell (Fig. 6*e*) both ends are usually rounded, but in forms about to leave the cell (*f*), and in free forms (*g*) the posterior end tapers to a point, while the anterior end is broadly rounded; several transition stages between the type shown in (*e*) and those shown in (*g*) are to be seen in our slides. The intracellular gametocytes averaged 7.1μ by 1.9μ, and the free vermicules varied according to their development, the largest being 13.4μ by 2.9μ and the usual size 11.5μ by 2.3μ. The cytoplasm stains much darker than is the case with the schizonts, and always has a pinkish tint which they never show; it is somewhat blotchy in appearance and the anterior end is always darkly stained. The chromatin may extend throughout the posterior half of the body or be collected into a mass of blocks and granules near the middle. It is not possible to differentiate between male and female gametocytes in our material, nor was there any indication of the occurrence of a process of maturation. The gametocytes must leave the cells very rapidly on the blood being drawn, because a considerable number of free forms were seen, which is remarkable in view of the haste with which smears were made. Of course, they may exist free in the blood, but this is not the case with other species of Haemogregarine which we have been able to observe in the living state. The proportion of schizonts to gametocytes varies in the different infections, those showing the most gametocytes being probably the oldest.

Type slide (*P. microlepis* No. 9c of our series) in the Australian Museum, Sydney.

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